

DETECTION AND QUANTIFICATION OF SELECTED VETERINARY ANTIMICROBIALS IN POULTRY EXCRETA

B. Gorissen^{1,2}, T. Reyns¹, M. Devreese², P. De Backer², J. Van Loco¹, S. Croubels²

¹Scientific Institute of Public Health, Department of Food, Medicines and Consumer Safety, Service of Chemical Residues and Contaminants, Rue Juliette Wytsmanstraat 14, 1050 Brussels, Belgium

²Ghent University, Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke, Belgium

Background

Due to the risk of antimicrobial resistance development, the Belgian Royal Decree concerning the eradication of *Salmonella* (C – 2007/22784) prohibits treatment of poultry with antimicrobials against zoonotic *Salmonella* spp. Nowadays, the unauthorized use of antimicrobials is determined through analysis of tissue samples of sacrificed animals. There is a need to develop a more animal-friendly method to detect illicit use of antimicrobials.

Objectives

Development and validation of an UHPLC-MS/MS method for the quantification of residues of β -lactams (amoxicillin (AMO) and phenoxymethylpenicillin), fluoroquinolones (enrofloxacin, difloxacin (DFX) and flumequine), sulfonamides (sulfachloropyridazine (SCP), sulfadiazine and sulfaclozine) in combination with trimethoprim (TMP), tetracyclines (chlortetracycline and doxycycline (DOX)) and for qualitative detection of polymyxins (colistin A) in samples of poultry manure.

Conduction of an animal experiment to gain insight into excretion of the selected compounds. The developed method was applied in the determination of target compounds in derived samples.

Background and objectives

Animal experiment

Twelve laying hens were equally divided into one control group and five experimental groups. For each antimicrobial class, one active compound was selected and administered by route of drinking water. Between every sample point, floor bedding, feed (and, during administration, medicated drinking water) were refreshed.



Animal experiment

Sample preparation

One g of poultry manure

+ 100 μ L IS working solution
+ 10 mL acetonitrile/McIlvaine buffer pH 3.6 (75/25, v/v)
Rotation, ultrasonication and centrifugation

One mL of supernatant

Ultracentrifugation
Filtration using Whatman™ PVDF 0.2 μ m filter

UHPLC-MS-MS

Acquity UPLC™ BEH C18 (100 \times 2.1 mm i.d., 1.7 μ m)
0.1% HCOOH in water (phase A) and 0.1% HCOOH in acetonitrile (phase B)
Xevo™ TQ-S triple quadrupole (Waters Corp., Milford, MA, USA)



Results

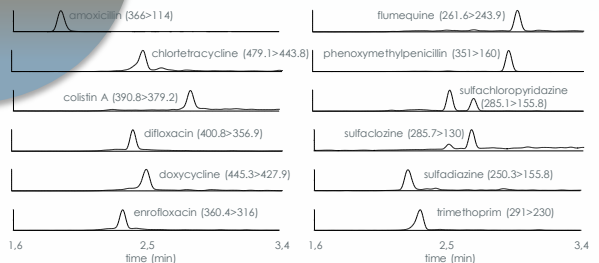


Figure 1. MS/MS chromatograms for a blank poultry manure sample spiked at 50 ng/g (LOQ)

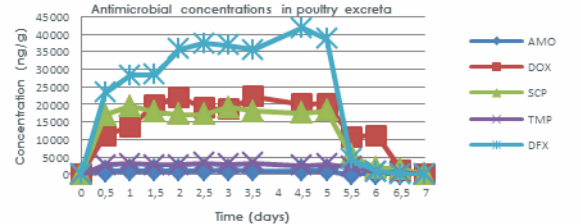


Figure 2. Results of an excretion study performed in laying hens, after oral administration of either DFX, SCP/TMP combination, DOX or AMO

Conclusions

- A quick and simple UHPLC-MS/MS method for determination of antimicrobials in non-invasive samples of poultry manure was developed and validated.
- The applicability of the developed method was tested on biological samples in a preliminary animal experiment; all studied compounds were successfully extracted (Fig. 2). Colistin A was detected until 1,5 days after the last treatment (LOD: 50 ng/g).
- The method is an animal-friendly alternative, suitable for high-throughput analysis in official control programmes to tackle the resistance development of *Salmonella* spp.